PB83-187930

Assessment of Maternal Toxicity, Embryotoxicity and Teratogenic Potential of Sodium Chlorite in Sprague-Dawley Rats

Ohio State Univ.
Columbus

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

1982

U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3.
EPA-600/J-81-363	JOURNAL ARTICLE	PB83-187930

4. TITLE AND SUBTITLE	5. REPORT DATE
Assessment of Maternal Toxicity, Embryotoxicity and Teratogenic Potential of Sodium Chlorite in Sprague-Dawley Rats	1982
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Daniel Couri, Carl H. Miller, Jr., Richard J. Bull, John M. Delphia and Elsayed M. Ammar	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Department of Pharmacology College of Medicine, Ohio State University Columbus, Ohio 43210	ABMC1A
	11. CONTRACT/GRANT NO.
	CR805643

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Research and Development Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	14. SPONSORING AGENCY CODE
	EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Environmental Health Perspectives Vol.46, 25-29, 1982

16. ABSTRACT

Groups of up to 13 pregnant rats were individually caged. Body weight, food and water consumption were recorded at days 1,8,15 and 22 of gestation and the dams were treated on days 8-15 with sodium chlorite, 0.1%, 0.5% or 2% in drinking water or by injection of 10,20, or 50 mg/kg IP or by gavaging with 200 mg/kg. To prevent ingestion of stillborn pups some dams were sacrificed at day 22. Other dams were allowed to deliver at term. Fetuses were weighed, measured and examined for soft tissue and skeletal malformations. Sodium chlorite, 20 or 50 mg/kg daily IP or gavaging with 200 mg/kg, caused vaginal and urethral bleeding. Doses of 10, 20 or 50 and 100% mortality of dams, respectively. No deaths were caused by sodium chlorite in the drinking water, but the dams' body weight, water and food consumption decreased during all treatments except 0.1% in the drinking water. Blood smears from the dams injected IP or drinking 2% sodium chlorite showed irregular, bizarre and ruptured erythrocytes. Injection of 10 or 20 mg/kg or drinking 2% resulted in decreased litter size and increased stillbirths and resorption sites. Drinking 0.1% or 0.5% sodium chlorite did not produce any significant embryotoxicity. With all treatments, no significant gross soft tissue or skeletal malformations were observed. Postnatal growth of the pups was not affected by any treatment of the dams during the gestation period.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	6
	20. SECURITY CLASS (This page)	22. PRICE
	Unclassified	

EPA-600/J-81-363
JOURNAL ARTICLE

Environmental Health Perspectives
Vol. 46, pp. 25-29, 1982

Assessment of Maternal Toxicity, Embryotoxicity and Teratogenic Potential of Sodium Chlorite in Sprague-Dawley Rats

by Daniel Couri,* Carl H. Miller, Jr.,* Richard J. Bull,* John M. Delphia† and Elsayed M. Ammar*

Groups of up to 13 pregnant rats were individually caged. Body weight, food and water consumption were recorded at days 1, 8, 15 and 22 of gestation and the dams were treated on days 8–15 with sodium chlorite, 0.1%, 0.5% or 2% in drinking water or by injection of 10, 20, or 50 mg/kg IP or by gavaging with 200 mg/kg. To prevent ingestion of stillborn pups some dams were sacrificed at day 22. Other dams were allowed to deliver at term. Fetuses were weighed, measured and examined for soft tissue and skeletal malformations. Sodium chlorite, 20 or 50 mg/kg daily IP or gavaging with 200 mg/kg, caused vaginal and urethral bleeding. Doses of 10, 20 or 50 mg/kg daily IP caused 0, 50 and 100% mortality of dams, respectively. No deaths were caused by soduim chlorite in the drinking water, but the dams' body weight, water and food consumption decreased during all treatments except 0.1% in the drinking water. Blood smears from the dams injected IP or drinking 2% sodium chlorite showed irregular, bizarre and ruptured erythrocytes. Injection of 10 or 20 mg/kg or drinking 2% resulted in decreased litter size and increased stillbirths and resorption sites. Drinking 0.1% or 0.5% sodium chlorite did not produce any significant embryotoxicity. With all treatments, no significant gross soft tissue or skeletal malformations were observed. Postnatal growth of the pups was not affected by any treatment of the dams during the gestation period.

Introduction

The chlorite ion is formed in water from chlorine dioxide treatment. Since chlorine dioxide is under investigation as an alternative to chlorine disinfectant in the U.S., the toxicological profile of chlorine dioxide and its products in water must be thoroughly investigated. Some hematologic aspects of chlorine dioxide and sodium chlorite toxicity have been assessed *(1–3)*. However, the health effects of chlorine dioxide or its products chlorite and chlorate have not yet been investigated during pregnancy. In this study we aimed to investigate the maternal toxicity, embryotoxicity and teratogenic potential of sodium chlorite in a pregnant rat model. Sodium chlorite was administered at various dose levels and by various routes of administration during the period of organogenesis in pregnant Sprague-Dawley rats. The parameters examined for assessment of chlorite toxicity in pregnant rats were maternal lethality or other toxic manifestation, changes in body weight, food and water consumption, fetal viability, size, weight, soft tissue and skeletal malformation.

Materials and Methods

Virgin female Sprague-Dawley rats weighing 268–303 g and fertile male Sprague-Dawley rats weighing 300–320 g were obtained from the Laboratory Supply Company, Indianapolis, Indiana.

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The rats were housed in the Ohio State University College of Medicine Vivarium and were given tap water and Purina Lab Chow ad libitum for one week for acclimation before the experiments began. During the course of the study rats were maintained at 55% RH, 73–74°F and a 12-hr light-dark cycle begining at 6:00 a.m.

Four female rats were mated with one male rat in the late afternoon and on the next morning female rats were checked for positive mating. The day of finding a vaginal plug and/or sperm in the vaginal smear examined under light microscopy was considered day 1 of gestation.

Rats verified for conception were weighed on day one and housed individually in cages provided with double distilled water and food ad libitum. The weights of pregnant rats as well as their water and food consumption were monitored at days 8, 15, and 22 of gestation. One group of rats was maintained on doubly distilled water till delivery to serve as the control. Other groups were given several treatments with sodium chlorite either by intraperitoneal injection, gavage or in drinking water during gestation period days 8–15. Sodium chlorite flakes ($NaClO_2$, MW 90.45) were obtained from Matheson, Coleman and Bell Manufacturing Chemists, Norwood, Ohio.

Sodium chlorite was administered to groups of rats IP at dose levels of 50, 20 and 10 mg/kg daily; by gavage at a dose of 200 mg/kg daily and in drinking water ad libitum at concentrations of 2%, 0.5% and 0.1%. Sodium chlorite solutions for drinking were replaced three times during the period of treatment (days 1, 3 and 5) with freshly prepared solutions. General health and toxic manifestation to the dams were observed during treatment and until delivery.

Some pregnant rats surviving treatment were allowed to deliver at term. Litter size, number of living and dead pups, weights of pups and crown-rump measurements were recorded. Offspring were examined for gross malformations and then killed with ether and divided into two groups. One group of pups was preserved in Bouin's solution for examination of soft tissue malformation under a dissecting microscope by the serial sectioning technique *(4,5)*. The other group of pups was fixed in 95% ethyl alcohol for skeletal examination after clearing of the specimens and staining with alizarin red *(6,7)*.

Since dams tend to cannibalize malformed or dead pups, other groups of pregnant rats were subjected to cesarean section before parturition at day 22. Dams were anesthetized with ether and the abdominal cavity was opened to expose the uterine horns which were longitudinally incised. Live, dead and resorbed fetuses were counted. Living fetuses were distinguished by the appearance of reflex motion after touching the fetus with forceps. Fetuses were then separated from the placenta, dried with filter paper, weighed and the crown–rump distance measured. As mentioned above, fetuses were prepared for gross, soft tissue and skeletal examination for malformation. The uterine horns were stained to reveal sites of early resorption *(8)*.

Blood samples were withdrawn from sacrificed dams by heart puncture and blood smears were stained with Wright stain to examine blood cells under light microscopy.

Postnatal growth was monitored by selecting six pups at random from each litter and weighing them at days 1, 8, 15, 22 and 29.

Statistical Analysis

A Hewlett-Packard 9825A and software Volume I was used to calculate significance according to Student's *t*-test or chi square. When the F test indicated the *t*-test could not be used with confidence, the normal curve and the larger standard deviation were used to estimate significance.

Results

Maternal Toxicity

The toxic effects and lethality of sodium chlorite administered to pregnant rats during gestation are summarized in Table 1. Sodium chlorite administered IP at a dose of 50 mg/kg produced profuse vaginal and urethral bleeding and 100% mortality within 2 days. The dams decreased in weight by 7%. However, the gain in weight during days 1–8, a no-treatment period, was 98%. The uterine horns were found to be implanted with an average of 12.4 embyros per litter. At a dose of 20 mg/kg IP, the mortality rate was 50%; vaginal and urethral bleeding as well as weight loss (19.5%) were also observed. At a dose of 10 mg/kg IP, sodium chlorite did not cause lethality or bleeding, but loss of weight was still observed. Effects of gavaging with 200 mg/kg sodium chlorite were similar to effects of 50 mg/kg IP. The various concentrations of sodium chlorite 2%, 0.5% and 0.1% in drinking water did not cause any deaths.

Effects of Sodium Chlorite on Dams' Body Weight, Food and Water Consumption

The changes in dams' body weights, food and water consumption are shown in Table 2. It is evident that the dams' body weight decreased during all treatments with sodium chlorite except 0.1%.

Table 1. Toxicity of sodium chlorite in pregnant Sprague-Dawley rats.

$NaClO_2$ treatment	Number of rats in group	Route of administration	Treatment period, gestation days	Toxic effects observed	Lethality, %
50 mg/kg	7	IP	8-10 or 16-17	Vaginal, urethral bleedings; loss of weight	100
20 mg/kg	10	IP	8-15	Vaginal, urethral bleedings; loss of weight	50
10 mg/kg	13	IP	8-15	Loss of weight	0
200 mg/kg	4	PO Gavaging	8-10	Vaginal, urethral bleedings; loss of weight	100
2%	10	PO Drinking water	8-15	Loss of weight	0
0.5%	10	PO Drinking water	8-15	Loss of weight	0

Table 2. Changes in dams' body weight, food and water consumption during and after treatment with sodium chlorite.

	Treatment on Gestation Days 8-15[a]			Recovery on Gestation Days 15-22[a]		
Treatment	Body weight change, g/rat	Food consumed, g/rat	Water consumed, ml/rat	Body weight change, g/rat	Food consumed, g/rat	Water consumed, ml/rat
Double distilled water (control)	27.20 ± 7.63	166.20 ± 10.66	353.60 ± 27.98	32.80 ± 1.24	113.20 ± 14.90	284.80 ± 19.29
2% $NaClO_2$ in drinking water	−72.50 ± 6.18[b]	54.50 ± 5.80[b]	63.22 ± 6.52[b]	98.00 ± 9.17[c]	118.50 ± 8.190[c]	276.11 ± 8.23[c]
0.5% $NaClO_2$ in drinking water	−18.75 ± 21.40[d]	112.75 ± 18.32[c]	224.37 ± 51.78[d]	86.71 ± 7.96[c]	139.50 ± 6.60[d]	264.87 ± 14.68[c]
0.1% $NaClO_2$ in drinking water	71.11 ± 16.60[d]	168.44 ± 3.19	237.55 ± 8.33[b]	14.44 ± 17.15[d]	147.11 ± 7.52[d]	292.22 ± 13.03[c]
10 mg/kg $NaClO_2$ IP	3.69 ± 6.25[b]	129.43 ± 10.07[d]	249.54 ± 10.97[b]	74.77 ± 9.92[d]	141.50 ± 6.27[d]	286.31 ± 9.21[c]
20 mg/kg $NaClO_2$ IP	−53.20 ± 7.58[b]	59.0 ± 10.89[b]	203.00 ± 26.25[c]	74.00 ± 28.59[d]	157.60 ± 11.15[d]	312.00 ± 25.72[c]

[a]Data represent means ± SE of 7-13 animals. On day 1, mean weight of rats in various groups ranged between 268 and 293 and the differences were not significant.
[b]$p < 0.001$.
[c]$p < 0.01$.
[d]$p < 0.05$.
[e]$p > 0.05$.

The increase in body weight under the influence of 0.1% sodium chlorite was significantly higher than the control group maintained on double distilled water. All sodium chlorite treatments whether in drinking water or by IP injection significantly decreased water consumption during the treatment period. Food consumption was decreased during all treatments except 0.1% sodium chlorite in drinking water.

During the period following the end of treatment until day 22 (recovery, Table 2) there was no significant difference in water consumption between treated and control rats. Food consumption was also increased for all treated groups, except the groups which drank 2% sodium chlorite or 0.1% sodium chlorite. During the recovery period the body weight gain for those groups which lost weight during treatment was significantly higher than the body weight gain in the control group.

Effect of Sodium Chlorite Treatment on Red Blood Cells of Dams

Blood smears from dams sacrificed at day 22 showed irregular blood cells, ruptured cells and hemolysis. This was observed only in rats which received 20 mg/kg IP and in those which drank 2% sodium chlorite.

Effect of Sodium Chlorite Treatment on Embryo and Fetus

The litter size, number of live and stillborn fetuses, the fetal weight and crown-rump measurements of litters delivered at term are shown in Table 3. With 2% sodium chlorite in drinking water, as well as with 10 mg/kg IP treatment of the dams, the litter size decreased and the number of stillbirths increased, but the changes were not statistically significant ($p > 0.05$) by the t test. The crown-rump distance was significantly decreased under all treatments. However the fetal weight was not significantly decreased. Table 4 shows the effect of sodium chlorite treatment on fetuses of rats sacrificed at day 22. It is evident that the higher concentrations of sodium chlorite increased the number of dead and resorbed fetuses and decreased both the number of live fetuses and the crown-rump distance. There were three whole litter resorptions out of five treated

Table 3. Effect of administration of sodium chlorite to pregnant Sprague-Dawley rats during day 8-15 of gestation on litters delivered at term.

Treatment	Litter size[a]		Crown-rump, length, cm[a]	Fetus weight, g[a]
	Alive	Stillborn		
Double distilled water (control)	9.80 ± 1.11	0	4.44 ± 0.07	7.24 ± 0.31
2.0% $NaClO_2$ in drinking water	6.5 ± 2.50	2.0	4.18 ± 0.05[b]	6.52 ± 0.22
0.5% $NaClO_2$ in drinking water	11.75 ± 0.75	0	4.30 ± 0.06[b]	6.54 ± 0.12
0.1% $NaClO_2$ in drinking water	9.80 ± 0.40	0.5	4.22 ± 0.02[b]	6.85 ± 0.16
10 mg/kg IP	8.86 ± 0.99	1.14	4.27 ± 0.05[b]	6.61 ± 0.22

[a]Data represent means ± standard error of 4 to 7 litters.
[b]$p < 0.05$.

Table 4. Effect of administration of sodium chlorite to pregnant Sprague-Dawley rats during day 8-15 on fetuses delivered at day 22 by cesarean section.

Treatment	No. of fetuses			Crown-rump length cm[a]	Fetus weight, g[a]
	Alive	Dead	Resorbed		
0.1% $NaClO_2$ in drinking water (control)	40[b]	1[b]	4[b]	3.66 ± 0.03	4.14 ± 0.65
0.5% $NaClO_2$ in drinking water	44[b]	1[b]	2[b]	3.87 ± 0.05[d]	5.23 ± 0.14
2.0% $NaClO_2$ in drinking water	37[b]	2[b]	21[b]	3.37 ± 0.10	4.59 ± 0.19
10 mg/kg $NaClO_2$ IP	58[c]	3[c]	7[c]	4.06 ± 0.04[d]	5.35 ± 0.14
20 mg/kg $NaClO_2$ IP	15[c]	0[c]	34[c]	—	—

[a]Data represent means of 4 or 5 litters.
[b]$p < 0.005$ by X^2.
[c]$p < 0.0001$ by X^2.
[d]$p < 0.05$ by t test.

Table 5. Postnatal growth of pups delivered to dams treated with sodium chlorite during days 8 to 15 of gestation.

Treatment	Postnatal day[a]					Postnatal mortality
	1	8	15	22	29	
Double distilled water (control)	42.8	113.8	209.4	274.3	456.7	0
2% $NaClO_2$ in drinking water	39.8	108.4	200.1	274.4	492.2	0
0.5% $NaClO_2$ in drinking water	38.2	102.4	209.0	326.7	484.5	0
10 mg/kg $NaClO_2$ IP	40.5	110.7	215.7	332.9	496.3	0

[a]Data represent mean weight in grams of 6 pups selected at random from each of 4 or 5 litters.

with 20 mg/kg IP, two litters out of five from dams drinking 2% sodium chlorite and one whole litter resorbed out of five dams given 10 mg/kg IP.

Effect of Maternal Treatment with Sodium Chlorite on the Postnatal Growth of Pups

The postnatal weights of six pups from each litter is shown in Table 5. There was no significant difference in the weight of pups delivered by control and treated dams.

Effect of Sodium Chlorite Treatment on Soft Tissues and Skeletal Formation of the Fetus

The incidence of soft tissue and skeletal malformations in the control group and sodium chlorite treatment was not significantly different and falls within the normal range of spontaneous malformation in rats.

Discussion

Sodium chlorite administered to pregnant Sprague-Dawley rats exerted toxic effects when given either

Table 6. Calculated daily dose of sodium chlorite administered to pregnant rats in drinking water.

$NaClO_2$ treatment	Sodium chlorite, mg/rat/day
Control	0
2%	212
0.5%	163
0.1%	34

by intraperitoneal injection, by gavage or in drinking water. The injection of 50 mg/kg or 20 mg/kg daily resulted in a cumulative toxic effect which caused 100% and 50% mortality, respectively. Gavaging of 200 mg/kg daily also produced 100% mortality. Injection of sodium chlorite as well as administration of sodium chlorite in drinking water in concentrations higher than 0.1% caused body weight loss and decreased food and water consumption during the treatment period, days 8–15 of gestation. However, after stopping sodium chlorite treatment, the body weight gain was more than the controls, probably as a compensatory mechanism. Decreased food intake continued even after cessation of treatment which may indicate a delayed toxic effect on the dams. Blood smears of rats receiving the higher concentrations of sodium chlorite, 20 mg/kg IP and 2% in drinking water, showed hemolysis of red blood cells.

The embryos and fetuses were affected by sodium chlorite treatment in higher concentrations administered either IP or in drinking water. The toxic effects on the fetus include decreased litter size, increased stillbirths and increased resorption of embryos. Whole litter resorption was observed in dams treated with the 2% sodium chlorite in drinking water and in those injected with 10 and 20 mg/kg IP. This, coupled with the observation of vaginal bleeding during treatment, may indicate an early abortifacient effect of higher doses of sodium chlorite. The toxic effect on the fetus may be due to hypoxia produced as a result of methemoglobinemia (*1-3*) and hemolysis. However, a direct toxic effect on the embryo cannot be excluded since results from this laboratory have shown that sodium chlorite is embryotoxic in the early stage of chick embryo development (*9*).

Teratogenic effects of sodium chlorite were not reported. We feel that expanded studies with sodium chlorite at other concentrations are needed to shed light on the embryotoxicity of sodium chlorite. The route of administration is crucial in assessment of the embryotoxicity of sodium chlorite as Table 6 indicates that the calculated amounts of sodium chlorite administered to rats daily were higher by the oral route than by injection. However, the injected sodium chlorite produced a higher maternal and embryotoxicity. Also, gavaging 200 mg/kg produced more toxicity than higher amounts given ad libitum in drinking water.

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